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TRICHODERMA SPP. AND METAL CHELATOR ENHANCED GROWTH AND YIELDS IN WHEAT PLANTS BY INCREASING ZINC AVAILABILITY

Sonia Saini

Department of Botany, Isabella Thoburn College, Lucknow (U.P) India.

ABSTRACT

A pot experiment of wheat was conducted with five different treatments viz. (I-Control, II-20 mg kg^{-1} Zn, III-50% compost extract, IV-50% extract+bioagent (Trichoderma spp.) and V-EDTA+Zn 20 mg kg^{-1}) to test the response of wheat plants grown in alluvial soil under warehouse condition. The growth attributes like plant height, fresh and dry matter yield enhanced with addition of compost extract, bioagent and EDTA. Number of tillers $plant^{-1}$, no. of inflorescence $plant^{-1}$, inflorescence length $plant^{-1}$, weight $inflorescence^{-1}$, no. of grains ear^{-1}, and weight per 100 seeds significantly increased in the plants treated with zinc in combination with compost extract, bioagent and EDTA. The value of total chlorophyll for control plants was 2.80 mg g^{-1} fresh weight, which were significantly increased by 5.3, 10.7, 14.2 and 23.2% on addition of zinc and its combination with different agents. The activity of catalase in shoot for control plants was 655 mg g^{-1} fresh weight, which was changed to 685, 705, 755 and 808 for II, III, IV and V level of treatments. The activity of amylase and peroxidase were also found to increase after addition of compost, bioagent and EDTA in leaves of wheat plants. The sugar, protein and proline content were significantly higher in the plants treated with different combinations.

KEY WORDS: Wheat, Reproductive yield, Catalase, Chlorophyll and Trichoderma.

Introduction

The growth and development of crop needs optimum ratio of micronutrient in soil. Deficiency or efficiency of these nutrients in soil is affecting the plant growth leads to severe loss in the productivity and yield. For proper growth and yield, it is necessary to maintain soil fertility. Many agricultural agencies and scientific organization suggested to farmer that how to maintain soil fertility and how to manage their loss. Here, study was focused on an essential micronutrients zinc, chelating agents, (ethylene diamine tetra acetic acid) and biofertilizer (*Trichoderma* spp). which functions as co-factor in many enzymes that participates in many metabolic and defense process. Zn deficiency leads to reduction in growth and yield. Chelating agents have been shown to desorb heavy metals from the soil matrix into the soil solution, and to facilitate metal translocation from roots to shoots. The effect may vary from plant and metal species. Chelating agents are frequently used to increase the bioavailability of heavy metals, thus enhancing their uptake by plants, though this may also decrease their biomass because of the toxicity. Chelators can mobilize soil nutrients and increase their bioavailability to the plants. The bioavailable metal content can be evaluated through the sequential extraction procedure. EDTA (ethylene diamine tetra acetic acid), a strong chelating agent, has been most extensively studied and continued to be for soil remediation due to its strong complexes-forming ability. The concept of biofertilizers was developed based on the observation that these micro organisms can have a beneficial effect on plant and crop growth (Davidson, 1988). One of the important genus for biofertilizer producers is *Trichoderma*, a fungus present in nearly all soils. *Trichoderma* spp. thrive in the rhizosphere and can also attack and parasitize other fungi. *Trichoderma* spp. have been known for decades to increase plant growth and crop yield (Lindsey and Baker, 1967, Chang *et al.,* 1986 and Harman, 2000), to improve crop nutrition and fertilizer uptake (Harman, 2000 and Yedidia *et al*., 2001) to speed up plant growth and enhance plant greenness (Harman, 2006), as well as to control numerous plant pathogens (Weindling, 1932, Shoresh and Harman, 2008 and Cuevas *et al*., 2005). A part of these effects may also be related to the fact that some *Trichoderma* spp. seems to hasten the mineralization of organic materials (Cuevas, 1991), thus probably releasing nutrients from soil organic matter. Positive effects on plant nutrition were also described for other organisms, and many soil bacteria may enhance the mineral uptake of the plant, as for example by the increased solubility of phosphate in the soil solution (Goldstein and Liu 1987). Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism uptake of nitrogen and protein quality; (ii) photosynthesis, chlorophyll synthesis, carbon anhydrase activity; reported that Zn-deficient plants reduce the rate of protein synthesis and protein content drastically. Mn is required for biological redo-system, enzyme activation, oxygen carrier in nitrogen fixation (Romheld and Marachner, 1995). Zinc deficiency in plants is most frequently corrected by application of Zn in soils. In most instances, Zn deficiency in crop species is corrected by applying 4.5 to 34 kg Zn ha^{-1} as broadcast $ZnSO_4$ (Martens and Westermann, 1991). But limited source of this compound feels a problem of future when the present sources become ended. The present investigation was undertaken to assess the effect of Zn singly and in combination with compost extract, bioagent (*Trichoderma spp.*) and EDTA on growth and certain metabolic responses of wheat plants.

Material and methods:

A soil-pot culture experiment was conducted under controlled glass house conditions at the Botany Department, Isabella Thoburn College, Lucknow during 2014 to study the effect of metal chelater (EDTA), bioagent (Trichoderma spp.) and various levels of Zn and their interaction with compost extract in alluvial soil on bio-chemical parameters, vegetative growth and reproductive yield of wheat plant. A pot experiment was conducted with five different treatments viz. (I-Control, II-120 mg kg^{-1} Zn, III-50% compost extract, IV-50% extract+bioagent (*Trichoderma spp.*) and V-EDTA+Zn 20 mg kg^{-1}) in alluvial soil under warehouse condition. In this experiment seeds of wheat (*Triticum aestivum* L.) were raised in soil filled in clay pots (15 kg size) amended with I-no treatments(control), II-Zn (20 mg kg^{-1}),III- 50% compost extract, IV- 50% extract+bioagent (*Trichoderma spp.*) and V-EDTA+Zn (20 mg kg^{-1}). For the determination of fresh and dry weight at two stages of growth after clear appearance of visible symptoms and growth differences, the plants were sampled thoroughly washed with running tap water and rinsed two or three times with distilled water to remove surface contamination. After rinsing the plant samples were gently blotted to wipe out the absorbed water

and separated into different plant parts and weighed quickly on balance to avoid excessive loss of water by evaporation. The dry weight of plants was determined after drying these fresh samples in pre heated oven at 70°C for 48 hours. The oven dried material was transferred to desiccator and weighed after cooling at room temperature. The dried plant material was also utilized for elemental analysis after wet digestion. Among the growth parameters, plant heights were measured at 45 and 120 DAT. Dry matter production per plant was recorded at the time of harvesting. Total number of tillers was counted in randomly selected three plants at 120 DAT. The main component of reproductive yield in wheat plants is number of inflorescence per plant, length of inflorescence per plant, number of grains per inflorescence. Number of grains per ear was calculated from three selected plants in each treatment. Mature 100 grains were selected randomly from each treatment and their weight was recorded in gram. Chlorophyll contents were measured by the method of Lichtenthaler, (1987). Carotenoids content were calculated on leaf fresh weight basis according to formula given by Duxbury and Yentsch (1956) and the results were expressed in fresh weight basis in mg g^{-1}. Protein content was estimated by the method of Lowry *et al.* (1951). Proline content was estimated calorimetrically by the method of Bates *et al.* (1973). Sugar content was determined by the method of Dubois *et al.* (1956). Catalase activity was assayed following to the method prescribed by Euller and Josephson (1927) with some recent modifications for the estimation of catalase activity by Bisht (1978). Activity was measured in terms of ml H_2O_2 hydrolyzed/g fresh weight. Peroxidase activity was estimated by using the method of Luck (1963). Amylase activity was assayed by the method of Katsuni and Fekuhara (1969). All the experiments were conducted in triplicates and data given in tables are average of the three replicates and statistically analyzed for the calculation of mean, standard error (SE), standard deviation (SD) and least significant difference (LSD).

Results

The value of Zn content in shoot for control plants was 9.45g g^{-1} DW which was significantly (p=0.01) increased by 53.4, 12.4, 17.6 and 90.4% in plants grown at II, III, IV and V levels of treatments. However, the value of Zn contents in roots of control plants was 13.5g g^{-1} DW which were changed to 19.2, 15.6, 17.2 and 25.5 g g^{-1} DW *at* II, III, IV and V levels of treatments respectively.(table 2). In the present study, shoot length for the control plants was 39.0cms which increased to 42.0, 48.0, 56.3 and 58.6 cms for II, III, IV and V level of treatments observed at 45 days. While at 120 days shoot length for the control plants was 92.8 cms which increased to 98.5, 101.5, 109 and 115.2cms for II, III, IV and V level of treatments. Fresh weight for shoot in control plants was 1.6 gm. Increase percentage was 6.2, 17.5, 34.3 and 12.5 % for II, III, IV and V level of treatments. Dry weight for shoot in control group was 7.0 gm. Increase percentage was 17.1, 40, 61.4 and 84.2 % for II, III, IV and V level of treatments.(table 1). Number of tillers $plant^{-1}$, No. of inflorescence $plant^{-1}$, inflorescence length $plant^{-1}$, No. of grains ear^{-1}, weight $Inflorescence^{-1}$ and Weight $seeds^{-100}$ significantly ($p<0.01$) increased up to V level of treatments in soil. Number of tillers for control was found to be 7.80 which was changed to 8.23, 8.98, 9.50 and 10.46 in wheat plants grown in II, III, IV and V level of treatments respectively. As compare to control, Number of inflorescence $plant^{-1}$ was increased by 15.8, 24.3, 34.2 and 53.6% at II, III, IV and V level of treatments. Inflorescence length $plant^{-1}$ for control was found to be 10.3 which were change to 11.5, 12.3, 13.6 and 14.8 for plants treated with II, III, IV and V level of treatments respectively. The value of number of grains per ear for control plant was 32.3 which were changed to 37.5, 44.5, 52.5 and 64.6 at II, III, IV and V level of treatments respectively. The value of 100 seeds weight for control was 1.48 g which were changed to 1.54, 1.73, 1.78, and 1.89 for plants grown in II, III, IV and V level of treatments.(table 4). The value of total chlorophyll for control plants was 2.80 which were significantly increased by 5.3, 10.7, 14.2 and 23.2% for II, III, IV and V levels of treatments. However, the value of chlorophyll a, for control plants was 1.80 which increased by 5.5, 13.8, 14.4 and 22.2% for II, III, IV and V Treatments. The value of Chlorophyll b, for the control plants was 1.00 which significantly changed to 1.05, 1.05, 1.06 and 1.15 for II, III, IV and V level of treatments. Synthesis of carotenoids significantly (p=0.01) increased with increasing concentration of Zn up to V level. As compared to control plant carotenoids content increased by 36.2, 53.4 and 58.6% at III, IV and V levels of treatments.(table 3). The activity of catalase was measured in term of H_2O_2 decomposed mg^{-100} fresh weight tissue. The activity of catalase in shoot for control plants was 655 which were changed to 685, 705, 755 and 808 for II, III, IV and V level of treatments. The activity of amylase was measured in term of ml H_2O_2 hydrolyzed mg^{-1} fresh weight. The activity of amylase in shoot for control plants was 3.85 which were increased to 3.95, 4.55, 5.26 and 5.63 ml H_2O_2 hydrolysed mg^{-1} FW for II, III, IV and V level of treatments. The activity of peroxidase was measured in term of H_2O_2 decomposed mg^{-100} fresh weight tissue. The activity of peroxidase in shoot for control plants was 6.56 which were increased by 15.2, 27.4, 50.7 and 44.8% for II, III, IV and V level of treatments. The sugar content was significantly higher in the seedlings treated with Zn than in the control. Sugar synthesis was different in different plant tissue. Therefore, the sugar contents in shoot were respectively 2.56, 2.86, 3.23, 4.00 and 5.12 at I, II, III, IV and V level of treatments. The value of protein for control plants was 112 which were significantly changed to 125, 136, 153 and 175 for II, III, IV and V level of treatments.. The Proline content was significantly higher in the seedlings treated with Zn than in the control. Proline synthesis was different in different plant tissue. Therefore, the Proline contents in shoot were respectively 2.56, 3.65, 4.18, 4.56, and 4.89 at I, II, III, IV and V level of treatments.(table 3)

Table 1: Effect of metal chelater (EDTA), bioagent (Trichoderma spp.) and various levels of Zn and their interaction with compost extract in soil on growth of wheat (*Triticum aestivum* L.) plants.

Parameters	DAS	Treatments				
		I	II	III	IV	V
Shoot Length (cm.)	45	39.0 ± 0.54	42.0 ± 2.08	48.0 ± 1.0	56.3 ± 0.30	58.6 ± 0.83
	120	92.8 ± 3.46	98.5 ± 2.36	101.5 ± 1.50	109 ± 4.04	115.2 ± 3.09
Fresh weight (g $plant^{-1}$)	45	1.60 ± 0.15	1.70 ± 0.17	1.88 ± 0.22	2.15 ± 0.32	1.80±0.45
Dry weight (g $plant^{-1}$)	120	7.0 ± 0.58	8.2 ± 0.69	9.8 ± 0.46	11.3 ± 0.17	12.9±0.52

I-Control, II-20 mg kg^{-1} Zn, III-50% compost extract, IV-50% extract+bioagent (Trichoderma spp.) and V-EDTA+Zn 20 mg kg^{-1},*- value significant at P<0.05 and **- value significant at P<0.01 levels; Parenthesis indicate percentage increase (+) or decrease (-) over control.

Table 2: Zn contents in plants after harvesting the wheat (*Triticum aestivum* L.) plants at maturity.

Parameters	Treatments				
	I	II	III	IV	V
Plant (g g^{-1} dry weight)					
Shoot	9.45 ± 0.17	14.5 ± 0.46*	10.63 ± 1.10	11.12 ± 0.06	18.0±1.23**
Root	13.50 ± 0.52	19.20 ± 2.00*	15.65 ± 1.09	17.20 ± 0.57	25.56±0.57**

I-Control, II-20 mg kg^{-1} Zn, III-50% compost extract, IV-50% extract+bioagent (Trichoderma spp.) and V-EDTA+Zn 20 mg kg^{-1},*- value significant at P<0.05 and **- value significant at P<0.01 levels; Parenthesis indicate percentage increase (+) or decrease (-) over control.

Table 3: Effect of metal chelater (EDTA), bioagent (Trichoderma spp.) and various levels of Zn and their interaction with compost extract in soil on biochemical parameters of wheat (*Triticum aestivum L.*) plants observed at 55 days.

Parameters	Treatments				
	I	II	III	IV	V
Chlorophyll a(mg g^{-1} FW)	1.80±0.23	1.90±0.05	2.05±0.02*	2.06±0.01*	2.20±0.11**
Chlorophyll b(mg g^{-1} FW)	1.00±0.08	1.05±0.02	1.05±0.05	1.06±0.03*	1.15±0.08**
Total Chlorophyll a(mg g^{-1} FW)	2.80±0.11	2.95±0.17	3.10±0.05*	3.20±0.11*	3.45±0.25**
Carotenoids(mg g^{-1} FW)	0.58±0.04	0.65±0.02	0.79±0.05*	0.89±0.05**	0.92±0.06**
CAT (ml H_2O_2 hydrolysed mg^{-1} FW)	655±14.43	685±20.20	705±8.66	755±2.88*	808±10.39**
Amylase(ml H_2O_2 hydrolysed mg^{-1} FW	3.85±0.15	3.95±0.08	4.55±0.20	5.26±0.15*	5.63±0.34**
Peroxidase(ml H_2O_2 hydrolysed mg^{-1} FW)	6.56±0.09	7.56±0.09	8.36±0.20*	9.89±0.22**	9.50±0.28**
Sugar(mg g^{-1} FW)	2.56±0.03	2.86±0.09	3.23±0.13	4.00±0.28*	5.12±0.06**
Protein (mg g^{-1} FW)	112±6.92	125±14.43	136±20.78	153±7.50*	175±14.43**
Proline(mg g^{-1} FW)	2.56±0.09	3.65±0.08	4.18±0.10*	4.56±0.03**	4.89±0.05**

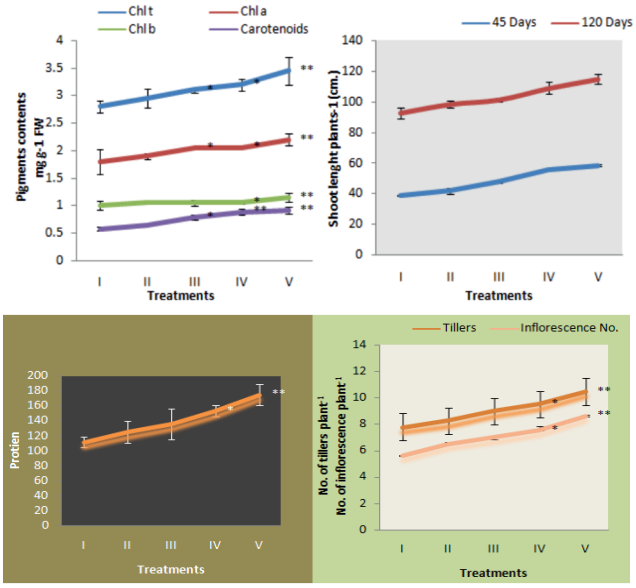
I-Control, II-20 mg kg^{-1} Zn, III-50% compost extract, IV-50% extract+bioagent (Trichoderma spp.) and V-EDTA+Zn 20 mg kg^{-1},*- value significant at P<0.05 and **- value significant at P<0.01 levels; Parenthesis indicate percentage increase (+) or decrease (-) over control.

Table 4: Effect of metal chelater (EDTA), bioagent (Trichoderma spp.) and various levels of Zn and their interaction with compost extract in soil on reproductive yields of wheat (*Triticum aestivum L.*) plants observed at 120 days.

Parameters	Treatments				
	I	II	III	IV	V
No. of tillers $plant^{-1}$	7.8±0.46	8.23±0.13	8.98±0.05	9.5±0.29*	10.46±0.27**
No. of inflorescence $plant^{-1}$	5.63±0.08	6.52±0.07	7.0±0.29	7.56±0.32*	8.65±0.38**
inflorescence length $plant^{-1}$	10.3±0.17	11.5±0.29	12.3±0.17	13.6±0.35*	14.8±0.46**
No. of grains ear^{-1}	32.3±2.40	37.5±1.32	44.5±1.44	52.5±1.44*	64.6±1.76**
Weight $inflorescence^{-1}$	2.75±0.1	3.03±0.55	3.28±0.12*	3.45±0.26*	3.65±0.20**
Weight $seeds^{-100}$	1.48±0.08	1.54±0.11	1.73±0.13*	1.78±0.23*	1.89±0.17**

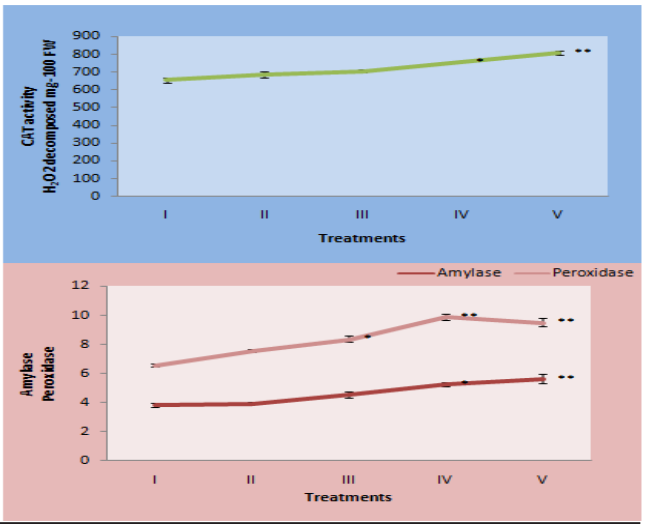
I-Control, II-20 mg kg^{-1} Zn, III-50% compost extract, IV-50% extract+bioagent (Trichoderma spp.) and V-EDTA+Zn 20 mg kg^{-1},*- value significant at P<0.05 and **- value significant at P<0.01 levels; Parenthesis indicate percentage increase (+) or decrease (-) over control.

Figure 1: Effect of metal chelater (EDTA), bioagent (Trichoderma spp.) and various levels of Zn and their interaction with compost extract in soil on shoot length and pigments contents, protein contents, no. of tillers and no. of inflorescence of wheat (*Triticum aestivum L.*) plants.



*-value significant at P<0.05 and **- value significant at P<0.0 levels; (standard error among triplicate).

Figure 2: Effect of metal chelater (EDTA), bioagent (Trichoderma spp.) and various levels of Zn and their interaction with compost extract in soil on activity of catalase, amylase and peroxidase of wheat (Triticum aestivum L.) plants.



*-value significant at P<0.05 and **- value significant at P<0.0 levels; ± (standard error among triplicate).

Discussion:

Zn content in shoot for control plants was 9.45g g^{-1} DW which was significantly (p=0.01) increased by 53.4, 12.5, 17.6 and 90.4% in plants grown at II, III, IV and V levels of treatments. However, the value of Zn contents in roots of control plants was 13.5g g^{-1} DW which was changed to 19.2, 15.6, 17.2 and 25.5 g g^{-1} DW at II, III, IV and V levels of treatments respectively (table 2). The increase in Zn supply also increased the Zn concentration in different plant parts as it has been reported earlier (Pandey et al. 2002). Shoot length for the control plants was 39.0cms which increased to 42.0, 48.0, 56.3 and 58.6cms for II, III, IV and V level of treatments. Fresh weight for shoot in control group was 1.6 gm. Increase percentage was 6.25, 17.5, 34.3 and 12.5 % for II, III, IV and V level of treatments. Dry weight for shoot in control group was 7.0 gm. Increase percentage was 17.1, 40, 61.4 and 84.2 % for II, III, IV and V level of treatments (table 1). Number of tillers $plant^{-1}$, No. of inflorescence $plant^{-1}$, inflorescence length $plant^{-1}$, weight $Inflorescence^{-1}$, No. of grains ear^{-1} and Weight $seeds^{-100}$ significantly ($p<0.01$) increased up to V level of treatments. No. of tillers for control was found to be 7.80 which was changed to 8.23, 8.98, 9.50 and 10.46 in wheat plants grown in II, III, IV and V level of treatments respectively. As compare to control, No. of inflorescence $plant^{-1}$ was increased by 15.8, 24.3, 34.2 and 53.6% at II, III, IV and V level of treatments. Inflorescence length $plant^{-1}$ for control was found to be 10.3 which were change to 11.5, 12.3, 13.6 and 14.8 for plants treated with II, III, IV and V level of treatments respectively. The value of no. of grains per ear for control plant was 32.3 which were changed to 37.5, 44.5, 52.5 and 64.6 at II, III, IV and V level of treatments respectively (table 4). Similar results were reported by Maralian (2009), Yilmaz *et al.* (1997), Seilsepour (2007) and El-Majid *et al.* (2000). Seilsepour (2007) found that the average grain yield increased by using Fe and Zn (317 and 330 kg/ha, respectively). Shahabifar and Mostashri (2002) have also reported that by consumption of 40 kg zinc sulphate, can increase wheat yield by as much as 473 kilograms per hectare. Researchers studied the effect of zinc resources on growth and yield of wheat showed that all the zinc resources, increases seeds yield (Dasalkar et al, 1992). Majidi and Malakoti (1998), stated that the use of zinc will increase wheat grain and nitrogen percentage significantly. This represents a positive role in enhancing the effects of zinc and also on yield and yield components of wheat (Header and Beringer, 1981). The value of total chlorophyll for control plants was 2.80 which were significantly increased by 5.3, 10.7, 14.2 and 23.2% for II, III, IV and V levels of treatments. The results of Zn application effect on pigment contents also reported by the Bassi and Sarma (1993). These results are in agreement with the results obtained by Rashid and Ahmed (1997) on broad bean plants. They found that, chlorophyll contents were increased by using foliar application with Zn (50 ppm). However, the value of chlorophyll a, for control plants was 1.80 which increased by 5.5, 13.8, 14.4 and 22.2% for II, III, IV and V Treatments. The value of Chlorophyll b, for the control plants was 1.00 which significantly changed to 1.05, 1.05, 1.06 and 1.15 for II, III, IV and V level of treatments. Hassanein *et al*. (2000) found that, spraying cow pea (*Vigna sinensis*) plants with 10, 50 and 250 mg/L of B or Zn caused high significant increases in the contents of chlorophyll (a) and chlorophyll (b). The activity of catalase in shoot for control plants was 655 which were changed to 685, 705, 755 and 808 for II, III, IV and V level of treatments. The activity of peroxidase in shoot for control plants was 6.56 which were increased by 15.2, 27.4, 50.7 and 44.8% for II, III, IV and V level of treatments. Tobbal (2006) and Gamal El-Gebaly *et al.* (2003) reported increased activity of CAT and POX enzymes in plants treated with Zn.The activity of amylase in shoot for control plants was 3.85 which were increased to 3.95, 4.55, 5.26 and 5.63 ml H_2O_2 hydrolysed mg^{-1} FW for II, III, IV and V level of treatments. The sugar content was significantly higher in the seedlings treated with Zn than in the control. Sugar synthesis was different in different plant tissue. Therefore, the sugar contents in shoot were respectively 2.56, 2.86, 3.23, 4.00 and 5.12 at I, II, III, IV and V level of treatments. Singh *et al*., (2007) and Manivasagaperumal *et al.,* (2011) also reported decrease in sugar content in wheat and cluster bean exposed to higher dose of Cu. The value of protein for control plants was 112 which were significantly changed to 125, 136, 153 and 175 for II, III, IV and V level of treatments. Tobbal (1999) reported that, the contents of soluble proteins in fenugreek and chickpea plants were increased in response to the treatment with Zn (100ppm) as foliar spraying. The Proline content was significantly higher in the seedlings treated with Zn than in the control. Proline synthesis was different in different plant tissue. Therefore, the Proline contents in shoot were respectively 2.56, 3.65, 4.18, 4.56, and 4.89 at I, II, III, IV and V level of treatments (table 3). The enhancement of proline level can be used as an indicator of the species tolerance to cadmium toxicity (Wu *et al.* 1995). Proline has been reported to play important roles in osmoregulation, protecting enzymes denaturization, acting as a source of carbon and nitrogen, stabilizing the machinery of protein synthesis, regulating the cytosolic acidity and/ or scavenging hydroxyl radical (Saradhi and Saradhi, 1991). Proline accumulation could probably also be related to a decrease in electron transport in plants under stress conditions (Venekamp, 1989).

Conclusion

The study showed that different treatments of $ZnSO_4$ had promotive effects on almost all of the growth, yield and qualitative parameters discussed. Application of Zn together with metal chelater (EDTA), bioagent (Trichoderma spp.) and compost extract have better response in terms of growth and yield compared to Zn alone.

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